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Self-Assembled Polymeric Membranes and Nanoassemblies on Surfaces: Preparation, Characterization, and Current Applications

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Biomembranes play a crucial role in a multitude of biological processes, where high selectivity and efficiency are key points in the reaction course. The outstanding performance of biological membranes is based on the coupling between the membrane and biomolecules, such as membrane proteins. Polymer-based membranes and assemblies represent a great alternative to lipid ones, as their presence not only dramatically increases the mechanical stability of such systems, but also opens the scope to a broad range of chemical functionalities, which can be fine-tuned to selectively combine with a specific biomolecule. Tethering the membranes or nanoassemblies on a solid support opens the way to a class of functional surfaces finding application as sensors, biocomputing systems, molecular recognition, and filtration membranes. Herein, the design, physical assembly, and biomolecule attachment/insertion on/within solid-supported polymeric membranes and nanoassemblies are presented in detail with relevant examples. Furthermore, the models and applications for these materials are highlighted with the recent advances in each field.

1. Introduction

Biomimetic membrane technologies represent a promising area of research to improve human health,[1–3] quality of life, and the environment.[4–6] Bioinspired approaches for membrane formation and the use of novel biological materials interfaced with stable synthetic materials constitute major opportunities for research and development in the area of biomimetic membranes.[7,8] The integration of relevant proteins with useful and accurate functions into stable polymer membranes allows the fabrication of smart nanoassemblies and active surfaces for a broad-spectrum of translational applications spanning from solar energy production and water purification to biomedical application for cancer treatment, microbiology, infectious disease, and regenerative medicine. Furthermore, polymer-based membranes and nanoassemblies are currently emerging technologies in drug screening and development (enzymatic arrays, bio-responsive devices, imaging diagnostics) as well as antimicrobial tools for medical treatments and research since they can help in exploring antibiotic kinetics and vaccination development.[9,10] Polymeric membranes and nanoassemblies are often employed on a surface, that can be a homogeneous solid support or a porous matrix, according to the final use the membrane is destined for. Membrane lateral mobility which largely depends on the flexibility and fluidity of the membrane is a key parameter to gain functionality.[11,12] These membranes outperform under various aspects their lipid counterparts, when it comes to mechanical stability, versatility, and tunable thickness.[13,14] Moreover, proteins with specific functions and conformations are inserted/attached into/onto relatively simple polymer membranes and assemblies to obtain bioactive surfaces.[15–17] The development of hybrid materials based on biological materials and synthetic materials aims 1 day to reach the complexity, efficiency, and responsiveness of model organ systems. Here, we present first the methods currently in use to prepare functional surfaces on solid or porous support and the biomimetic strategy to produce and modify synthetic membranes and nanoassemblies with biomolecules (enzymes, proteins, transporter, DNA) and catalysts in order to gain an appropriate function and dynamic. While bilayers are mimics of biomembranes, we added polymer brushes and self-assembled monolayers with appropriate molecular properties allowing the combination with biomolecules because biomimicry can be understood not only by mimicking the architecture of a bio-membrane but in addition, by providing a matrix/template which allows biomolecules to be attached/inserted and still preserve their functionality. We illustrate the main sophisticated high-resolution analytical techniques used to explore the physicochemical characteristics of the membranes and consequently the specificity and applicability of planar membranes at the nanoscale. Considerable efforts have been directed to the development of "smart" polymer nanoassemblies that respond

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to physical or chemical stimuli such as the presence of specific molecules or redox chemistry, which overcome the lack of functionality of lipid membranes.[13,14] However, we decided not to treat in depth such stimuli responsive systems as they have been extensively described elsewhere.[18,19] Furthermore, we present hybrid biologic–synthetic nanoassemblies used to improve the systems and achieve functional applications. Finally, we include a discussion on the fundamental and practical challenges and future steps for future improvements and development of solid-supported bio-hybrid membranes.

2. Planar Membrane Fabrication

Several methods have been developed to obtain functional planar membranes on a support, which consists of two or more steps according to the complexity of the system. The first step consists in the functionalization of the surface with chemical anchors and subsequent physical or chemical growth of the membrane that can be obtained via two grafting techniques. The second step includes the attachment/insertion of the active molecules on/inside the synthetic planar membranes/nanoassemblies with the aim to induce the designated functionality.

2.1. Grafting Methods

Grafting techniques are employed to cover surfaces with polymer brushes and polymeric nanoassemblies. In general, the concept of grafting includes a wide variety of compounds on a surface, such as polymeric micelles or vesicles, planar polymer assemblies at the air/liquid interface, diblock melts, and polymeric chains tethered to a surface.[20,21] To achieve the polymer attachment, a "bottom-up" or a "top-down" approach is possible.

2.1.1. "Grafting to" Method

The "top-down," known as "grafting to," consists in the prepolymerization of macromolecules in different architectures such as linear, hyper-branched, dendrimeric, and 3D networks polymers that are subsequently covalently bonded to an activated support, commonly silica- or gold-based (**Figure 1**A).[22] In order to deposit the macromolecules on the surface, a chemical modification of it is necessary, to allow the immobilization of the polymers/network. The surface is modified by the introduction of functional groups that can react with the polymer/nanoassembly that has to be attached. The surface modification spans from chemical functionalization with small molecules that serve as initiators or reactive braces for self-assembled monolayers (SAM). Common functionalization is based on highly reactive molecules that undergo high yielding chemistry, such as EDC/NHS,[23] "Click,"[24] thiol–ene,[25] and Sn2 chemistry.[26] Since the polymer chains are produced in advance, high control over the brush thickness is achieved by controlling the chain length. Moreover, the control over the chemical functionality of the brushes is easy to obtain as the chains are produced in solution and only subsequently bonded to the surface. The main drawback of this approach consists in the low packing density of the polymer brushes due to the high steric hindrance of the already attached brushes. Besides SAMs that spontaneously assemble on the surface in high packing densities, the reactive polymer chain has to diffuse through

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Cornelia G. Palivan received her Ph.D. degree in Physics at the University of Bucharest, after a two years research stage at the University of Geneva. Currently Professor in Physical Chemistry of the University of Basel and member of the Swiss Nanoscience Institute, she has as main scientific interests the development of functional bio-artificial systems that interface biomolecules with supramolecular synthetic assemblies (nanoparticles, polymersomes or solid-supported planar membranes). Such hybrid bio-artificial systems provide optimum conditions for complex reactions at the nano/micro-scale and support applications in domains such as medicine, catalysis, food- or environmental sciences.

Wolfgang Meier studied Chemistry at the University of Freiburg and received his Ph.D. degree in Macromolecular Chemistry in 1992. In 1996, he was appointed as a lecturer in Physical Chemistry at the University of Basel where he received his "Habilitation" in 1998. In 2001, he was appointed as a professor at the International University of Bremen; since 2003, he has been a Professor of Chemistry at the University of Basel and vice-director of the Swiss Nanoscience Institute. His main research interests are in the field of hierarchical self-assembly of functional polymers and polymer–protein hybrid materials.

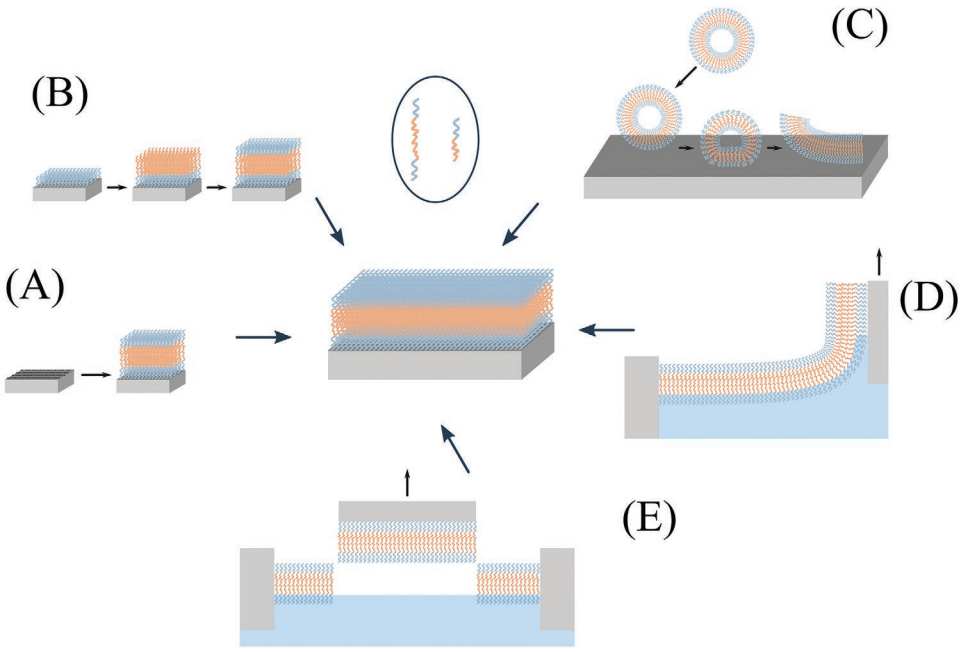

Figure 1. Scheme of different solid-supported polymer membrane preparation methods. A) "Grafting to" the surface, B) "grafting from" the surface, C) fusion of vesicles, and D, E) Langmuir monolayer transfers: Langmuir Blodgett and Langmuir-Schaefer.

the already attached chains, and the packing of these increases the steric barrier against new approaching polymer chains. To allocate more polymer chains, these have to stretch out into a straight coil conformation, which is entropically unfavored. For this reason, the density of the polymer layer is limited by the length of the polymer itself, thus high packing densities can hardly be reached.[27] Recently, this issue has been tackled by drop coating a triblock polymer brush composed of tert-butyl 2-((2-bromopropanoyloxy)methyl)acrylate-*b*-poly(ethyleneglycol) methylethermethacrylate-*b*-*N*-(3,4-dihydroxy-phenethyl) methacrylamide)-*g*-poly(2,2,3,3,3pentafluoropropylacrylate) ((*t*BBPMA-*b*-PEGMEMA-*b*-DOMA)-*g*PPFA), in which the anchors were the catechol moieties in the DOMA block that efficiently attached to the ITO substrate.[28] Another example that exploits "sticky" moieties is an ABA triblock in which the lateral A groups are composed of quaternarized 2-(dimethylaminoethyl) methacrylate (qDMAEMA) and the B block is a hydrophobic flexible backbone with poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) grafted on it for antifouling activity.

The "grafting to" method generally relays on thinner and more flexible chains, that react with the surface in high yielding reactions, which are commonly "click" reactions (Figure 1A).[24,29] In fewer cases, "Click" chemistry has also been used in the grafting of bulkier macromolecules like dendrimers or hyper branched polymers to a surface.[30] Several cycles of grafting are however required to obtain coverage of the surface since the steric hindrance provided by the bulk of the dendritic coronas does not allow a one-step coverage, such in the case of a PAAc polymer on a PE modified surface.[31] Moreover, this technique allows the decoration of surfaces with biopolymers, such as sugars or proteins.[32] For example, a polyurethane film was decorated with collagen and chitosan polymers for antiviral applications.

2.1.2. "Grafting from" Method

The "bottom-up" approach that allows the fine-tuning of the brush functionality and density is the "grafting from" method (Figure 1B). The "grafting from" method consists in the functionalization of a surface with a reactive monomer/initiator that promotes in situ polymer growth.[33] Since homogeneous chain length is preferred for the formation of homogeneous membranes, living polymerizations are preferred in respect to free radical ones or polycondensations. However, since the polymerization occurs directly from the surface, brush length, the average molecular weight the dispersity and the grafting density are complex to characterize, and require the use of highly specific analytical tools, which will be described in the following sections. Many polymerization techniques are used to achieve homogeneous polymer brushes,[34] such as RAFT, ATRP, NMP, and ROMP.[35–38] Since monomer diffusion through the growing chains is much easier than polymer diffusion, the drawbacks in packing density encountered in the "grafting to" approach are avoided here and the brush formation is much faster.[34] The main advantage of the "grafting to" methods is that the polymer brushes can be designed to carry functional groups for molecule anchoring, or that allow further functionalization and crosslinking (**Table 1**).[39] The anchoring of biopolymers like cellulose, chitin or sugars has also been in the focus recently, allowing the production of functional biomembranes from renewable sources.[40] For example, a bilayer composed of chitosan and alginate has proven high efficiency and mechanical stability as filtration membranes.[41] The production of biopolymers from bio monomers instead requires their chemical modification to introduce a reactive moiety, such as an acrylate or vinyl group, which allows them to retain their chemical structure and function unaltered while

Table 1. Advantages and disadvantages of preparation methods of solid-supported membranes.

Membrane preparation	Advantages	Disadvantages
Grafting to	High control over thickness, functionality	Low packing density
Grafting from	High packing density	Low control over homogeneity in thickness, challenging characterization
Fusion/rupture of vesicles	Simplicity, spontaneous surface related no special equipment	Membrane defects, low reproducibility, limited number of surfaces to apply on, in infancy
Langmuir Blodgett and Langmuir Schaefer	More dipping modes	Special equipment, high degree of cleanliness
Pore spanning membranes	Studying of transmembrane processes	Stability, membrane defects

being transformed into a macromolecule. In case of cholesterol, addition of a methacrylate moiety was necessary for the polymerization on a functional glass surface, as for amino acid modified with vinyl groups.[42,43] Multistep polymerization for the formation of ABA polymer brushes grants access to membrane like structures.[44,45] Since the membranes are covalently bonded to the surface, they possess enhanced mechanical stability and undergo degradation much slower compared to their free-standing counterparts, which is a necessary characteristic for long-term applications.[13] For instance, a triblock composed of two blocks of zwitterionic sulfobetaine (SBMA) and glycidyl methacrylate grafted polymer brushes on planar surfaces also serve as functional supports to self-assembled lipid membranes to mimic the extracellular membrane.[46,47]

2.2. Membrane Preparation on Homogeneous Solid Support

2.2.1. Fusion of Vesicles

Lipid bilayers are often obtained by liposome fusion on a surface.[48] This process, which consists in the deposition of the liposomes on the support that fuse in a bilayer film, is a highly reliable method to obtain lipid-based defect-free membranes with high reproducibility.[49] Unfortunately, when the liposome fusion method is applied upon polymersomes to generate polymer membranes, structural defects within the membrane appear and they show scarce reproducibility in their characteristics (Figure 1C).[13,50] One of the main obstacles for polymer vesicles, namely polymersomes, to merge into a planar membrane, is their higher mechanical stability compared to liposomes, that prevents them to open up and merge as easily.[51] Moreover, liposomes present stronger Van der Waals interactions with the substrate compared to polymersomes, which enhance their spreading on a surface compared to their polymeric counterpart.[52] Another important factor preventing a rapid deformation and rupture of the polymersomes to generate planar solid-supported membranes is the higher bending modulus than in the case of liposomes. Therefore, polymersomes require higher attractive interaction to deform to conform to the surface than liposomes. Another important factor which hampers the opening of a polymersome and the organization of the polymer on a surface is the loss in entropy given by the loss of hydration of the polymeric chain and the decrease in conformational freedom derived by the packing within the membrane (Table 1). In fact, besides a complex scenario of parameters like temperature, surface chemistry, polymer chemical composition, critical osmotic pressure of the polymersome, ion strength, and pH of the internal and external environment of it, the vesicle fusion process is also extremely dependent on the size and dispersity of the polymersomes, which is harder to control than the aforementioned parameters.[49,53,54] Moreover, the fusion of polymersomes relays on the delicate imbalance between all these forces, further including surface charge density and hydrophobicity.[55] To clarify, if on one hand fusion of liposomes occurs under mild conditions, instead, the opening of a polymersome is triggered by enhanced hydrophilicity of the surface, as in the case of PDMAEAMA$_4$-PBMA$_{66}$-PDMAEMA$_4$ or PB-PEO vesicles, for which a surface with enhanced hydrophilicity was used.[50,56] In other cases, high temperatures are also used, as for poly(trimethylene carbonate)-*b*-poly(L-glutamic acid) (PTMC$_{22}$-*b*-PGA$_{14}$), which required a temperature higher than 40 °C to fuse. Besides high temperatures, polymer vesicle fusion also can require high external osmotic pressures, which trigger the dehydration of the vesicle lumen.[56] This is the case for poly(butadiene)-*block*-poly(ethylene oxide) (PB-PEO), for which the addition of 1.5 M of NaCl was used to increase the surface of the vesicles to induce strain and trigger their spreading and fusion on the support. Moreover, the incubation was led at 45 °C, showing that this method is more efficient above the physiological conditions. For these reasons, the polymer vesicle fusion method is rarely used for polymer-based membranes, which are obtained by the methods described in the coming paragraphs.

2.2.2. Mono/Bilayer Deposition

The Langmuir Blodgett technique (LB), associated also to its close relative Langmuir Schaefer technique (LS), is frequently used to attach ordered layers of surface-active molecules on a solid support (Figure 1D,E).[57,58] The LB method consists in spreading a block/co-polymer at the air/oil–water interface in a Langmuir through, vertically dipping and then removing the solid support while increasing the surface pressure, which allows the molecules to self-assemble in ordered layers. In this way, artificial defect-free biomimetic membranes are obtained, as in the case of poly(dimethylsiloxane-*b*-2-methlyoxazoline) (PDMS-*b*-PMOXA).[59] By dipping the support once, a monolayer deposition is achieved, and subsequent repetitions of dipping and extracting can form bi to multi-layered membranes known as layer-by-layer membranes (LBL). The thickness of the membrane can be thus fine-tuned depending on the number of dipping cycles and the length of the polymer chains. In general,

polymer membranes are either composed of monolayer in the dry thickness of a couple of nanometers, or of several micrometers thickness. Through the LB technique, the gap between these two can be filled via the ordered layer by layer deposition. In the case of asymmetric polymers, in important role in membrane formation is also the directionality of the membrane formation, as for the case of PEG_{45}-*b*-$PMCL_{101}$-*b*-$PDMEAEMA_{27}$ in which only the unidirectional double "up-up" transfer yielded a membrane with a dry thickness of 8 nm, namely the double of the 4 nm of the dry monolayer thickness.[60] The LS differs from the latter by the dipping mode, which occurs horizontally.[61] Lipoic acid functionalized p(butadiene)-*b*-p(ethyleneoxide) (LA-*b*-PB-*b*-PEO) triblocks were deposited via the LB technique to form a monolayer, and a highly ordered low defect bilayer was achieved by adding PB-PEO-OH via the LS technique.[60,61] Blockcopolymers are also used for the formation of active surfaces, for which a poly(*N*-isopropylacrylamide-*co*-*N*-2-thiolactoneacrylamide) (PNIPAM-*co*-TlaAm) was first compressed to a homogeneous film and the subsequent horizontal dipping of an activated silica substrate lead to the transfer of it to the solid support.[62] By mixing block copolymers and lipids, hybrid membranes become also accessible via layer deposition techniques, and permit the formation of domains or homogeneous membranes according to the miscibility of the lipid with the polymer.[63] Between the two techniques, the LS techniques reaches higher degrees of order within the membrane, yet is less versatile than the LB technique. For instance, the LB technique provides two dipping modes, which can be combined into sequences. When using this approach with an asymmetric ABC block polymer, the film thickness of the dry and wet state can be fine-tuned.[60] By controlling the surface pressure on the through, high control over the assembly and the membrane was achieved, and high packing densities have been reached by just increasing the surface pressure without drifting away from the monolayer assembly conditions.[64] Asymmetric membranes have also been reported by targeting the balance between surface pressure and surface chemistry.[13,65] In this case, an asymmetric ABC block has to be employed, in which the hydrophilic A and C block result to be immiscible and will for this reason assemble without mixing.[66]

Poly(ethyleneglycol)-*b*-p(γ-methyl-ε-caprolactone)-*b*-p(2-dimethylaminoethylmethacrylate) (PEG_{45}-*b*-$PMCL_{101}$-*b*-$PDMAEMA_{27}$) monolayer and bilayer membranes were obtained this way, achieving an asymmetric functional membrane for facilitated protein insertion.[60] Moreover, it is possible to transfer directly free-standing membranes without causing major damage or the rupture of the membrane.[67] The disadvantage of using the LB deposition is the high sensitivity of the Langmuir through itself, which requires long times and an extremely high degree of cleanliness (Table 1). For the latter, the complete absence of pollutants such as dust and small particles is fundamental to obtain defect-free membranes and thus hampers the scale-up of the membranes for industrial applications.

2.3. Pore Spanning Membrane

One of the main drawbacks on homogeneously solid-supported membranes and brushes is the loss of capability in mimicking physiological conditions (Table 1). The problem arises in the stiffness of the solid support, which might prevent the diffusion of the proteins through the membrane and their spontaneous positioning due to steric hindrance.[63] These processes can instead be studied well with free-standing membranes, which however lack of mechanical stability and decompose over the time span of hours.[13] Bringing together solid-supported and free-standing membranes opens the way to mechanically stable membranes which can be also studied in physiological conditions, as parts of the membranes are free-standing over the pores.[68] The technique is mostly used for lipid-based membranes, and employs porous organic or inorganic material as support.[69] The formation of pore spanning membranes can be approached with various methods. One of them consists in the preformation of lipid giant unilamellar vesicle (GUV), which are then spread the pores of the solid support.[70] Upon heating above the phase transition temperature, the lipid giants start to reorganize as a membrane. For polymers, brushes have been employed, as for example PMMA brushes tethered to porous silica which serve as a strong support for the insertion of membrane proteins, and permits the fine tuning of protein density throughout the membrane.[71] The insertion of proteins is made possible by the local removal of the steric hindrance of the solid support within the membrane spanning areas. For this reason, this local removal of the support's steric hindrance leads to the facile insertion of membrane proteins, and facilitates the study of transmembrane processes.[72] $PMOXA_{12}$-$PDMS_{54}$-$PMOXA_{12}$ was used as the building block for a pore spanning membrane over a carbonate etched support, covered in a thin Au layer and functionalized with photoreactive acrylate groups to ensure the tethering of the membrane.[3] The most common technique of obtaining pore-spanning membranes consists in the painting of amphiphilic molecules, both lipids or block-copolymers on the solid support, by employing organic solvents which have to be evaporated eventually.[73] However, when it comes to painting polymer membranes, the necessary use of organic solvents such as chloroform or toluene typically leads to the denaturation of the membrane proteins that have to be inserted.[74] To overcome this issue, $PMOXA_7$-$PDMS_{60}$-$PMOXA_7$ was solubilized in decane, which is protein-friendly, and let self-assemble on the support assisted by an auto painting membrane chamber.[74–76] The residual biocompatible solvent allows the direct insertion of membrane proteins, such as gramicidin, while ensuring high mechanical stability, which means longer membrane lifetimes. However, the polymer membranes spanning on the pores still retain the mechanical properties of freestanding membranes, resulting in brittle and short-lived membranes. Thus, the main issues encountered in pore spanning membranes are similar to the membranes obtained by vesicle fusion: presence of defects combined with not optimized coverage and delamination of the film.[77]

2.4. Biomolecules Incorporation into Planar Membranes

The design of a functional surface, requires various properties of the membrane, such as thickness, flexibility, charge, and membrane density, depending on the desired application (**Figure 2**A).[11,12] The mechanical properties largely depend

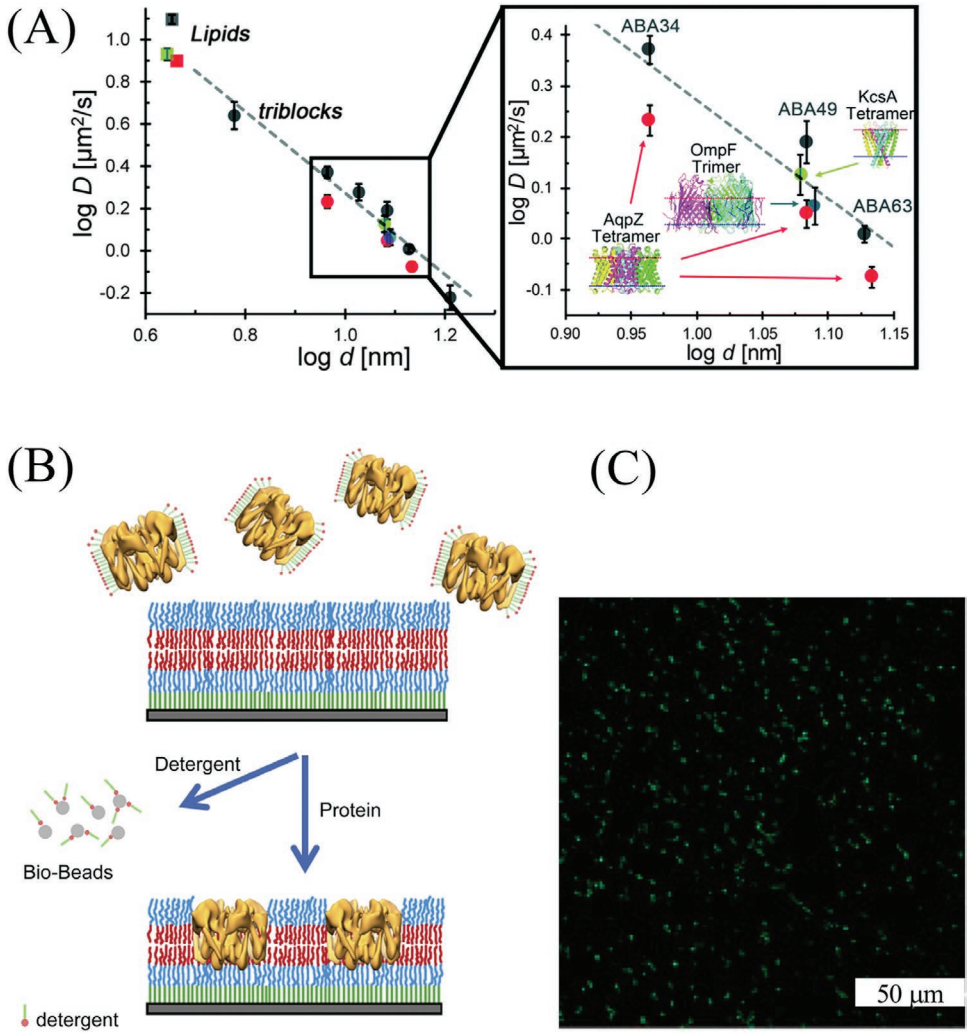

Figure 2. A) Log-log plots displaying the dependence of the diffusion coefficient *D* relative to the membrane thickness *d*. Reproduced with permission.[12] Copyright 2015, American Chemical Society. B) Schematic representation of the membrane protein MloK1 into solid-supported polymer membranes using Bio-Beads. C) CLSM micrograph of the successful incorporation of MloK1. Reproduced with permission.[59] Copyright 2014, Elsevier.

on the chemical composition of the copolymer and the length of the hydrophobic block used to form the membrane. The combination of chemical and physical structure of a polymer influence the lateral mobility of block copolymers membranes: membranes consisting of PMOXA and PDMS with different membrane thicknesses (6–20 nm) and different block architectures (diblock and triblock, PMOXA-*b*-PDMS-(*b*-PMOXA)) have been extensively investigated due to their fitting mechanical properties and biocompatibility (Figure 2A).[11] The lateral mobility of the block-copolymer within the membrane allows the successful insertion of membrane proteins, opening the way to synthetic investigations of natural processes (**Table 2**). The interdigitation and entanglement of the amphiphilic block copolymers chains have been determined to be the main factors contributing to the decrease in lateral diffusion, whereas the high fluidity and flexibility of the hydrophobic block (e.g., PDMS) have been found to be the key-factor for the incorporation of membrane proteins.[12] Because the activity of membrane proteins relies on their molecular stability and structural flexibility, determined by the tertiary and quaternary structure, the synthetic membrane has to provide a strong yet adaptable supporting matrix to retain the protein's structure. According to this, the functional incorporation of gramicidin, a small biopore with 2.5 nm height, has been successfully inserted in membranes up to 13 nm thickness and its functionality was fully preserved.[78,79] Nevertheless, the large mismatch between the membrane thickness and the size of the membrane protein, highly affects their insertion, mobility, and activity (Figure 2A). Consequently, parameters such as polymer hydrophobicity, membrane fluidity, and flexibility are essential for the insertion of biomolecules and preservation of their functionality. By finely regulating the physicochemical characteristics (chemical structure and composition) of the molecular block copolymers, membranes with a large variety of properties can be obtained, and the resulting supramolecular structures possess extraordinary mechanical and chemical stability.

Table 2. Insertion of biomolecules into planar polymer membranes.

Biomolecule	Polymer(s)	Membrane preparation	Biomolecule insertion
Gramicidin	PMOXA-PDMS-PMOXA[79]	Film rehydration	DMSO:EtOH 1:1
Laccase	PEG-PMCL-PDMAEMA[58,60]	LB	Adsorption
Tyrosinase	PEG-PMCL-PDMAEMA[60]	LB	Adsorption
Glucose oxidase	PGMA/PHEMA[85]	Grafting from	Covalently bound
HRP C	PHEMA[86]	Grafting from	Covalently bound at lysine
Alpha hemolysin	PB-PEO[72]	LB-LS	Direct immersion
MIoK1	PMOXA-PDMS[59]	LB-LS	Bio-Beads
Proteorhodopsin	PMOXA-PMDS-PMOXA,[98] PEG-PDPA-PSS[99]	Film rehydration	Dialysis, pH change in solution
OmpF	PMOXA-PDMS,[63] PMOXA-PDMS-PMOXA[12]	LB, film rehydration	Added at LB, Added at rehydration
AqpZ and AQP0	PMOXA-PDMS-PMOXA[12]	Film rehydration	Added at rehydration
Aldolase	PNIPAM-TIAa[62]	Deposition	Covalent bonding points
β-galattosidase	Poly[9,9-dioctylfluorene-co-thiophene][64]	Compressed to a film	Air–water interface

However, it is important to take into account also the nature of the biomolecules in terms of hydrophobicity or hydrophilicity in order to generate functional biointerfaces. The insertion process of the membrane proteins within the membrane is based on burying the hydrophobic amino acid residues in the hydrophobic part of the membrane, while the hydrophilic residues face the aqueous side and/or the hydrophilic part of the membrane.

2.4.1. Noncovalent and Covalent Binding of Proteins to Surfaces

Biomolecules can bind the surface of planar membranes mainly via two different approaches which principally diverge on the binding energy: physisorption or chemisorption. Physisorption is based on a non-specific and reversible interaction of the biomolecules with the membrane whereas chemisorption is based on irreversible chemical binding of the biomolecules to the membrane by modified end-groups of the amphiphilic block copolymers forming the membranes. For example, the versatile enzymes laccase and tyrosinase were physically attached to asymmetric membranes composed of PEG-*b*-PMCL-*b*-PDMAEMA on silica.[58,60] Such hybrid membranes on solid support were utilized to biosense and detoxify phenol derivatives. The stability and accessibility of enzyme activity as well as influence of molecular properties of polymer membranes on the enzyme immobilization served to optimize the biosensing activity of such functional membranes. Enzyme adsorption on polymer monolayers or bilayers was influenced by electrostatic interactions at the enzyme–polymer interface. Both laccase and tyrosinase maintained their enzymatic activity upon adsorption onto the polymer layers. However, depending on the type of polymer films (e.g., monolayer or bilayer) their activity changed and the enzymes on the bilayer specifically showed a greater activity for both enzymes.[60] However, the solid-supported polymer membranes prepared by a "grafting from" approach are not desirable for membrane protein reconstitution since the block copolymers covalently immobilized to the surface have a low lateral mobility, which prevent biomolecules to be inserted. To avoid physical coupling between the bilayer and the solid support, which could perturb the functionality of the inserted integral protein and interfere with the membrane dynamics, the creation of space between the membrane and the solid support is of critical importance. A space of several nanometers between the membrane and the substrate can be obtained by using tethers, cushions or brushes.[80–82] Polymer brushes directly attached to a surface and thus acting as spacers are mainly used for the development of biosensing, antimicrobial or antifouling surfaces due to their biocompatible properties and ability to covalently tether active compounds, such as peptides, enzymes or assemblies to different surfaces. "Grafting from" and "grafting to" are the typical forms of attachment.[83,84] For example, organic electrochemical transistors were fabricated from the conducting polymer PEDOT:PSS, while polymer brushes of PGMA and PHEMA were integrated into the devices as a scaffolding to anchor glucose oxidase (GOx).[85] The covalent functionalization of polymer brushes with glucose oxidase showed a high electrical performance and long-term stability without compromising the electrical performance of the PEDOT:PSS channel.[85] Moreover, using horseradish peroxidase C (HRP C) as a model enzyme and PHEMA as a model support, it has been explored how immobilizing an enzyme on a densely functionalized brush impacts activity and stability.[86] Covalent immobilization was achieved by highly functionalizing the PHEMA with N,N′-disuccinimidyl carbonate (DSC) in order to produce a dense monolayer of HRP C. The results indicate a loss of activity of the enzyme due to structural changes resulting from nonspecific interactions between the enzyme and the DSC activated brush suggesting the need to balance covalent immobilization while maintaining a passive surrounding.

2.4.2. Membrane Protein Insertion

Membrane proteins can be inserted into polymer membranes either during the membrane formation process or after the membrane is formed. To prove the successful incorporation,

a variation in electrical conductance during channel protein insertion has to be established as for the unequivocal functional incorporation of alpha hemolysin into a polymer membrane based on PB-*b*-PEO copolymers attached on patterned gold electrodes.[72] The membrane was assembled on a gold electrode using consecutive LB and LS transfers followed by addition of alpha hemolysin. This approach allowed a permanent and functional insertion of alpha hemolysin, as confirmed by a flow of ions across the membrane. Biobeads mediated membrane protein insertion has been also applied for protein insertion into planar solid-supported membranes (Figure 2B,C).[59] More specifically, nucleotide modulated potassium channel MIoK1 proteins were inserted into the PDMS-*b*-PMOXA membrane on solid support by using bio beads to force the protein insertion.[59] The controlled addition of bio-Beads is used to destabilize the membrane and to act as a driving force for membrane protein insertion into the polymer membrane attached to various solid substrates.[59]

2.4.3. Polymer–Lipid Hybrid Membranes for Protein Loading

Another approach that combines the robustness of polymeric membranes with properties of natural membranes, such as lateral diffusion and the formation of rafts, is to prepare hybrid membranes from amphiphilic block copolymers and phospholipids on solid substrates. For example, when PDMS-*b*-PMOXA diblock copolymers with different hydrophobic block lengths were mixed at different ratios with various lipids (1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE), and 1-palmitoyl-2-oleoyl phosphatidyl-ethanolamine (POPE), the LB technique generated monolayers revealed different morphologies with distinct domains of lipid- and polymer-rich phases after transfer to transparent substrates.[63] The ability to control insertion and location of membrane proteins due to the specificity of such domains makes hybrid films ideal candidates for applications where specific spatial functionality is required.[63] The LB and LS deposition are powerful techniques for the transfer of biomolecules onto ordered films, leading to an easy insertion within the targeted domains.[59] Both copolymers and block polymers function as supporting membranes for proteins, and lipid/polymer membranes are also accessible via this technique. For instance, PNIPAM-TlAa was deposited to promote aldolase insertion providing covalent bonding points without the use of further membrane crosslinking.[62] Poly[9,9-dioctylfluorene-*co*-thiophene] was used in combination with stearic acid for the immobilization of β-galattosidase: all three components were deposited at the air water interface and compressed to a film, which was subsequently transferred to a solid support.[64] More focus has been put onto transmembrane proteins and their interaction with hydrophobic domains. However, since the solid support provides steric hindrance for the protein to diffuse freely and thus hampers the observation of the proteins in their native environment, sometimes a gel layer or brushes are placed as spacers between the membrane and the solid support.[87,88] Generally known as cushions, they promote protein diffusion and relieve the steric hindrance given by the stiffness of the solid support.[63] They are obtained with the aforementioned grafting techniques. Polyelectrolyte (PE) brushes have been in the focus as they show strong interaction with lipid bilayers while preventing the solid support to interact with cytosolic domains of membrane proteins, as in the case of poly([(2-methacryloyloxy)ethyl]trimethylammonium chloride) (PMETAC), which was used as a support to characterize enzyme accessibility and membrane protein motion in planar cell membrane bilayers.[89] Also, by supporting a lipid bilayer with a PE brush yielded the same conductance as a back lipid bilayer.[90] Contrary to vesicle fusion, this method is suitable for block polymers, and paves the way to hybrid membranes that are able to support a higher number of functional biomolecules.

2.4.4. Bioinspired Polymer Membranes

Bioinspired polymer membranes can also spontaneously self-assemble in diluted aqueous conditions (e.g., water, buffer) to form vesicular architectures (polymersomes or GUVs, depending on their size), which provide compartmentalization for in situ reactions. Several membrane proteins have been successfully reconstitute into polymersomes membranes such as outer membrane protein F (OmpF),[63] proteorhodopsin (PR),[90,91] complex I, and AquaporinZ (AqpZ)[92,93] via detergent-mediated reconstitution,[93] electroformation,[94,95] peptide-induced fusion,[95] microfluidic jetting,[96] or spontaneous swelling.[97]

The compartments architecture (polymersomes, GUV) allows both the encapsulation of hydrophilic molecules such as enzymes and the confinement of hydrophobic ones within their hydrophobic domain. The selection of a particular amphiphilic blockcopolymer has to match the specificity of the biomolecules, and the intrinsic conditions of the desired application.[18] When it comes to directed protein membrane insertion, in which the proteins have to be inserted in only one direction, the asymmetry of amphiphilic block copolymers with different hydrophilic and hydrophobic domains is a key factor supporting the biofunctionality of active surfaces with desired orientations.[98] For instance, asymmetric polymersomes from an ABC block copolymer based on poly(ethyleneglycol)-poly(diisopropylaminoethyl methacrylate)-poly(styrenesulfonate) (PEG-PDPA-PSS) allowed the specific orientation of the light-activated proto pump proteorhodopsin (PR) due to the two different hydrophilic blocks (PEG and PSS). This asymmetric membrane allowed to achieve a directional and functional insertion of a membrane transport protein enabling an unidirectional exchange of solutes in the wanted direction (**Figure 3**A,B).[99] The reconstitution of the membrane protein into polymersomes has been made with the help of a proteorhodopsin-green fluorescent fusion protein (PR-GFP).[100] GFP guided the orientation during the insertion process into preformed vesicles due to its hydrophilic nature, which impedes passage through the hydrophobic part of the membrane.[101] Additionally, GFP's fluorescence allowed to detect the protein in the resulting assemblies. A further advance in guiding the orientation of the proteins across the membrane can be realized by equipping polymersomes with genetically or chemically modified membrane proteins, which have specific molecules or amino acids at desired locations. In the case of channels, besides pore permeability, these modifications

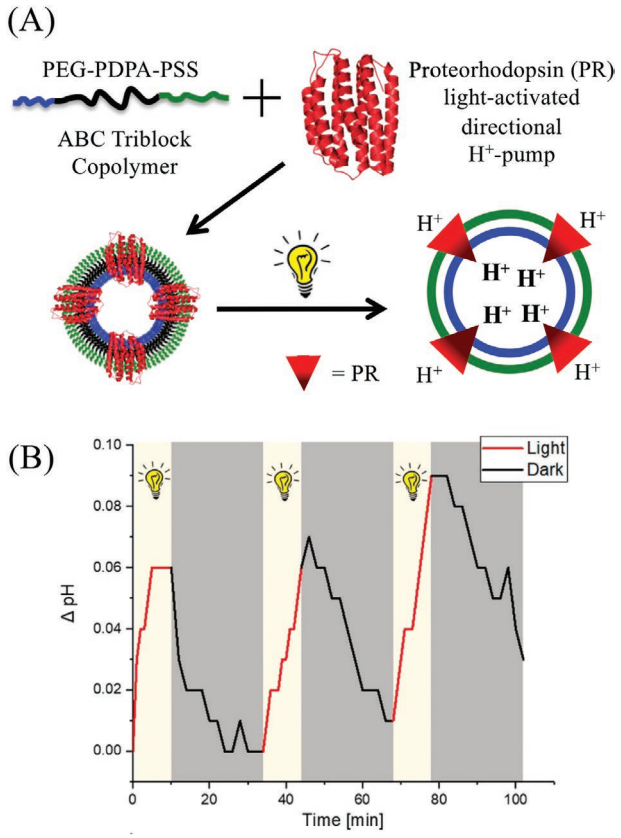

Figure 3. A) Simplified scheme of the self-assembly of an ABC triblock copolymer with PR. Adapted with permission.[99] B) Light induced bio-functionality of the PR inserted into polymersomes. Reproduced with permission.[99] Copyright 2019, American Chemical Society.

influence substrate selectivity and induce membrane responsiveness. For example, OmpF was chemically modified with a pH-responsive "molecular cap" (cyanine5-hydrazide) that serves as a "gate" inside the polymer membrane.[102] This is the first example of a chemical modification of OmpF that results in tuning the pore cutoff size (from 600 to 240 Da) and the molecular selectivity. Another elegant way to tune the pore size through OmpF proteins is to functionalize them with pH responsive peptides, which are able to block or liberate the pore according to the external pH.[103]

2.5. Self-Assembled Nanostructures on Surfaces

Self-assembly of polymers into 3D nanostructures such as micelles or polymersomes support the design of drug delivery systems, nanoreactors, and sensors.[104,105] In the case of nanoreactors, they exert their function by the introduction within their cavity of an active molecule or biomolecule and the use of a permeable membrane to allow a molecular flow of substrates and products through the polymersome's membrane.[103] Self-assembled polymers allow the better preservation in their lumen of enzymes, which are protected from external environmental conditions and yet are fully active in situ, in much higher controlled and specific conditions than when the enzymes are free in solution and subjected to proteolytic attack of eventual denaturizing agents. By immobilizing such catalytic polymersomes on a solid surface functional surfaces decorated with self-assembled supramolecular nanostructures are obtained.[78] The immobilization of the nanostructures enhances the versatility of the system, in which the nanoassemblies are less prone to mechanical stress, and it provides a higher control over the functionality of the surface.[106] The immobilization of nanostructures is performed either via non-specific binding, or by covalent or supramolecular bonding.[107] Among the three, the latter is preferred as it allows achieving higher control over the binding topology and distribution, as it has been reported by exploiting strain promoted click chemistry and thiol–ene reactions.[108] The metal-free highly reactive moieties (cyclooctine with azide) allowed the facile decoration of the PMOXA-PDMS-PMOXA assemblies on the surface. The surface was thus decorated with a reactive moiety that was able to recognize a binding site on the nanoassembly or nanoparticle. The bonding has been thus regulated via covalent attachment as in the case of the click reactions,[109] but also via biotin/streptavidin[110] for molecular recognition attachment or via DNA recognition.[111] The immobilization can be performed directly on the hard support, as in the case of cyclodextrin-based polymersomes covered by a biotin functional poly(acrylic acid), which is bonded on a streptavidin surface.[112,121–123] More common are the interactions of polymersomes with a soft functional surface composed of amphiphilic polymers or lipids.[113] In general, an amphiphilic bilayer is spread on a solid support, and the heads of the lipids are functionalized with an active molecule which acts as the recognition site.[113] Alternatively, molecular anchors can be employed, like cholesterol or aliphatic chains.[114,115] Choosing what kind of chemistry is required, as in the mobility of the bonds, bonding strength and reversibility are application dependent: for vesicle arrays and high throughput systems, stable anchored vesicles are required, on the other hand, to investigate vesicle dynamics, mobile structures need to be built.[116]

3. Characterization Methods of Functional Surfaces

A broad range of surface analytical methods are used to provide a detailed and quantitative understanding of the properties of artificial planar membranes. These methods allow a deep investigation of defects within the membranes, lateral homogeneity, translational fluidity, bilayer thickness, molecular orientation of bio-molecules as well as their packing densities (**Table 3**). Planar supported membranes, which are formed on hydrophilic solid supports including mica, silicon oxide or glass can be investigated by a combination of atomic force microscopy (AFM),[117] optical microscopy,[118] quartz crystal microbalance (QCM),[119] and surface plasmon resonance (SPR).[120] In order to determine the protein activity, electrophysiology is often employed.[121]

3.1. Atomic Force Microscopy

AFM provides information on the directionality and density of proteins once they are incorporated into the bilayer through

Table 3. Summary of characterization methods for solid-supported membranes.

Characterization technique	Properties assessed	Surface type	Advantages	Disadvantages
Atomic force microscopy	Various physical forces, elasticity, height differences, physical/chemical characteristics	Any kind	3D surface profile, atomic resolution, molecular forces, molecular interactions	Limited vertical range, influence of the tip, no high throughput
Quartz crystal microbalance with dissipation	Adsorption, desorption, decomposition, mass changes per unit area	Formed in situ	Less expensive than optical methods, high throughput screening, real-time, label-free technique	Longer response time, mass deposition in QCM measurements is higher than the optical measurements due to bound water molecules
Surface plasmon resonance	Thickness, refractive index, binding kinetics (K_{on} and K_{off})	Functionalized on chip	Relatively small amount of sample needed, faster response time, real-time, label-free technique	Very high sensitivity, sensitive to temperature
Brewster angle microscopy	Light polarization change	Film at air–water interface	Real time imaging, label-free technique, simple	Highly specific technique, resolution limitations
Ellipsometry	Dielectric properties	Any kind but dry	Accurate measurements of ultra-thin films of thicknesses below 10 nm	Low throughput screening, in situ measurement
Fluorescence microscopy	Fluorescence	Any kind	Live imaging, fast, simple	Fluorescent labeling required
Electrophysiology	Changes in voltage or electrical current, membrane potential	Membranes with inserted channel proteins	Recording of large-scale electrical signals	Higher level of background noise
Electrochemical impedance spectroscopy	Conductance, capacitance, resistance	Membranes with inserted channel proteins	Non-invasive and label-free technique	Complex data processing

their more hydrophobic/hydrophilic domains.[117] Furthermore, by applying loading forces to the AFM tip, biological samples can be perturbed to provide insights into protein assembly. AFM allows the understanding of the flexibility and the conformational changes of extrinsic domains as well as the intramolecular interactions that stabilize the proteins into the bilayers. High-speed atomic force microscopy (HS-AFM) can also be used to visualize function-related conformational changes of single soluble proteins and to study rafts,[122] pore forming toxins,[123] antibody–antigen interactions and membrane protein interactions[124] with nanometer spatial resolution and millisecond temporal resolution. However, although AFM offers good spatial resolution, it is worth to consider that also the application of small forces close to 10–12 N on compressible fluid membranes can induce molecular rearrangements and bilayer deformations. AFM, for instance, was used to confirm the incorporation of α-haemolysin (αHL) into solid-supported membranes based on PB-PEO polymer bilayer.[88] The AFM scan of pure PB-PEO indicated a smooth surface with an average roughness of about 0.9 nm. After incubation with αHL, the AFM image of PB-PEO indicated reconstitution in the polymer of the solid-supported membrane. The observed head sizes of 5.5–7.3 nm and channel sizes of 1–2 nm agreed well with αHL inserted in lipid membranes.[125,126] Furthermore, polymersomes based on disulfide-functionalized $PMOXA_{20}$-*b*-$PDMS_{75}$-*b*-$PMOXA_{20}$, with and without aquaporin-Z, were spread on gold-coated solid substrates with the help of covalent bonding of disulfide groups of polymer with gold (**Figure 4**D).[127] Remarkably, the successful deposition of AqpZ-containing polymersomes onto alumina with pores of 55 nm, was able to preserve the natural functionality of the protein. Thanks to the ability of AFM to sense and apply forces with high accuracy, AFM-based force spectroscopy (AFM-FS) has become an excellent tool to study solid-supported membranes. In the case of polymeric membranes, AFM-FS has become a very valuable approach to measure interactions and mechanical properties of membranes at the nanoscale with high spatial and force resolution.[128,129] Although AFM has been widely used to image the surface structures of micro-/nano-scale, it is also a force sensor with high sensitivity. AFM-FS is able to provide valuable information about the intramolecular and intermolecular interactions not only in polymer systems but also in bio-macromolecules and supramolecular systems, giving information on mechanical properties of single polymer chain, the interfacial conformation and adhesive energy of polymers, interaction between macromolecules and small molecules, and the direct measurement of intermolecular forces. [130–132]

3.2. Quartz Crystal Microbalance

Other methods besides the ones mentioned in Section 3.1 that can be directly applied to silicon- or glass-based chips are QCM-based methods, that function without the use of fluorescence probes and allow the studies of affinities and kinetics of receptor-ligand binding in supported membranes. Quartz crystal microbalance with dissipation (QCM-D) is a technique that measures adsorbed mass to surfaces by tracking the change in resonance frequencies of the surface that is oscillated via piezoelectric excitation.[119] QCM permits to directly detect molecular recognition and adsorption events on the surface of electrodes coated with Langmuir films, LB films, and SAMs. It easily exhibits measurable frequency changes due to mass

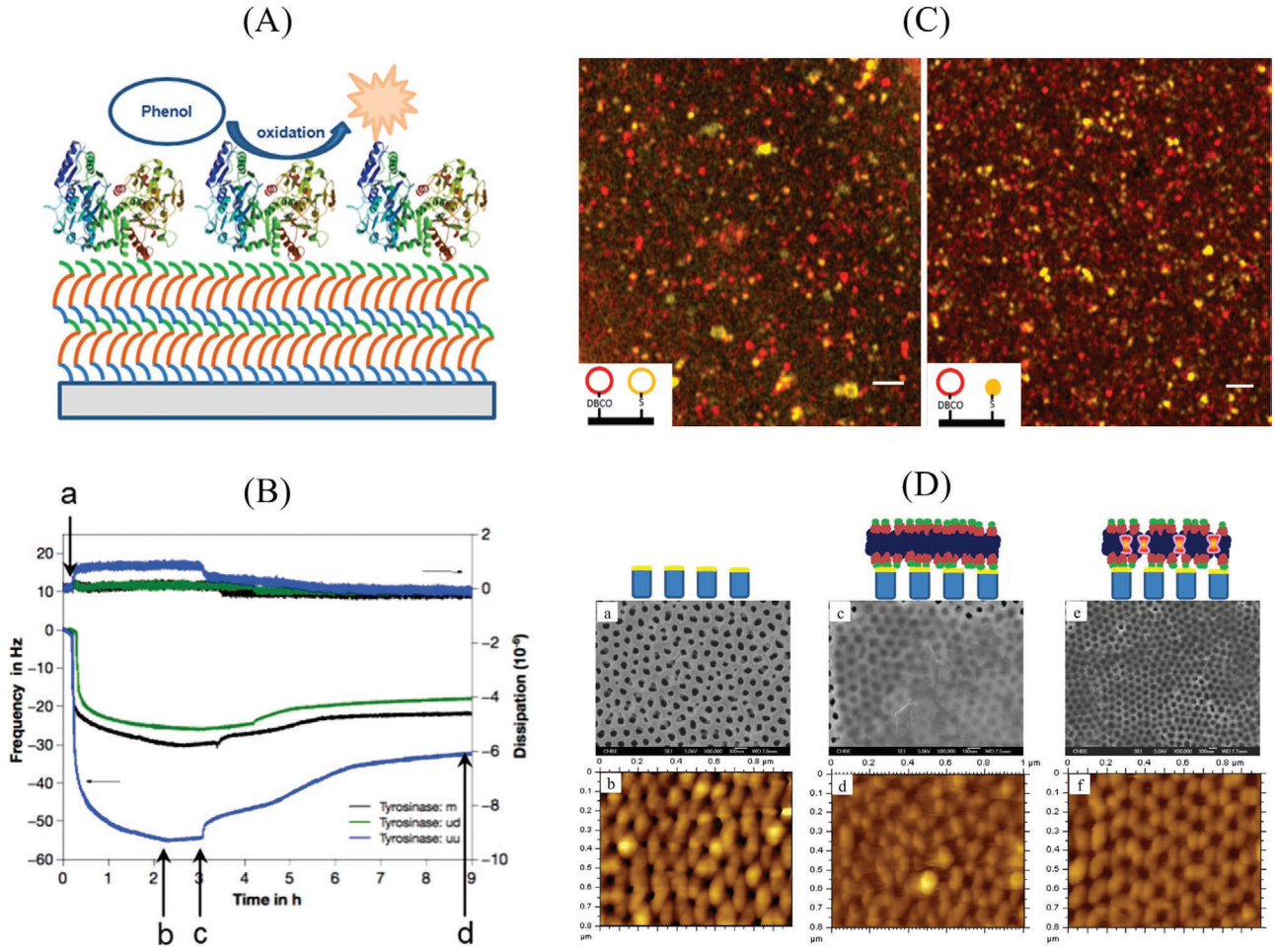

Figure 4. A) Schematic representation of tyrosinase immobilized on solid-supported polymer membrane. B) QCM graph of tyrosinase a) system stabilization, b) tyrosinase adsorption, c) incubation, and d) desorption. Adapted with permission.[60] Copyright 2018, American Chemical Society. C) CLSM micrographs of: co-immobilization of polymersomes (left); co-immobilization of micelles and polymersomers (right). Adapted with permission.[136] Copyright 2019, American Chemical Society. D) FESEM and AFM micrographs of a,b blank, c,d polymer membrane, and e,f AqpZ incorporated within polymer membrane. Reproduced with permission.[127] Copyright 2012, Elsevier.

changes on the electrode at the nanogram scale.[133] Different types of solid-supported polymer membranes (monolayer and bilayer) were studied by QCM-D, to calculate of the mass of the adsorbed enzyme on the membrane surface (Figure 4A,B). Changes in frequency (mass) and dissipation during adsorption, incubation, desorption of laccase, and tyrosinase on different polymer films were investigated.[134,135]

Another strategy for laccase immobilization on various conducting surfaces, which rely on ionic coordination chemistry involving —COO— terminal groups present on the protein, has been studied to determine the surface coverage as the Δm/MW ratio and the number of enzymes adsorbed per area of the polymer film.[134]

3.3. Surface Plasmon Resonance

A suitable method to characterize membranes and functional membranes and to obtain direct evidence of polymer adhesion on a planar surface is SPR spectroscopy which requires metallic substrates (e.g., Ag and Au) and a specialized chemistry to stabilize the bilayers.[120,137,138] SPR biosensors allow the in situ investigation of reversible molecular interactions and give evidence on the specificity of the interactions, binding affinity, binding levels, dissociation and association rate constants, and several key thermodynamic parameters, including entropy, enthalpy, and activation energy.[139–143] For instance, bovine rhodopsin incorporated into an egg phosphatidylcholine bilayer deposited on a silver film, allowed a tight binding and activation of its associated G-protein (transducin) as established by SPR.[144]

3.4. Brewster Angle Microscopy

The topography of thin films on a dielectric substrate can be explored by using Brewster Angle Microscopy (BAM). One of its applications consists in the visualization of the monolayer

at the air–water interface, which helps to determine the homogeneity of the surface layer. This technique is however limited to nonsupported Langmuir monolayers, and in order to obtain a solid-supported system as the ones described within this review, the formed film has to be transferred via LS or LB techniques.[145] BAM allows observation of the coexistence of domains of different phases in monolayers based on differences in membrane thickness and transitions between phases where differences in molecular orientation with respect to the water surface can be detected by changes of anisotropy.[146] Monolayer formation, phase transition, and protein–lipid phase separation within protein or protein-polymer mixed monolayers can be observed conveniently by the BAM technique.[147] By recording the parallel polarized reflectance of the sample at the Brewster angle of the substrate, the technique allows the real-time visualization of Langmuir monolayers.[118] Specifically, the lateral organization of the films, including formation of domains and phase separation can be easily examined.[118] For instance, hybrid polymer-lipid films based on amphiphilic copolymers PDMS-*b*-PMOXA were investigated, where BAM was able to resolve larger domains composed of saturated lipids and larger polymers, yet failed to describe with sufficient accuracy smaller domains, which arise with "liquid" unsaturated lipids mixed with the same polymer.[90] Monolayer state, compressibility, and phase transitions were analyzed on a Langmuir trough for three diblock copolymers (having 16, 37, and 65 PDMS units) and four lipids (DPPC, DPPE, DOPC, and POPE). Changes in monolayer morphology and domain formation were monitored by BAM. Phase separation occurred as a result of the different sizes of the polymer and states of the liquids in the mixtures, suggesting that the size and the number of various domains can be finely tuned by changing the component ratios and by modifying their length and stiffness. Different molecules or small changes within a molecule such as the molecule's length or presence of a double bond can alter the monolayer's lateral organization that is usually undetected using surface pressure-area isotherms. BAM permits the successful visualization of monolayers at interfaces by allowing label-free real-time images of fully hydrated films. Interactions that only resulted in minor changes of area-surface pressure isotherms still resulted in significant changes of the lateral film organization. Thus, the use of optical microscopy provides important additional insights to better understand the potential impact of macro-molecules, drug or drug carriers and other NPs on biologically relevant systems.

3.5. Ellipsometry

Another quantitative method for the determination of thin-film thickness is the ellipsometry.[137] By using this technique it is possible to get information regarding lateral uniformity, phase separation, bilayer thicknesses, and ligand–receptor binding interactions giving evidence in membrane structure and dynamics.[148] For instance, ellipsometry has been used to verify whether a lipid material desorbs from the mica surface.[149] Furthermore, the layer thickness of an engineered thiol-modified surfaces was determined by spectroscopic ellipsometry. Ellipsometry measurements revealed an increase in layer thickness after surface modification with thiols (1.3 nm) and after coupling of the PEG spacer (2.3 nm) suggesting an efficient strategy for coupling soft polymeric nano-architectures to functionalized surfaces.

3.6. Fluorescence Microscopy

Fluorescence microscopy has been also a major analytical technique for studying planar bilayers on solid substrates. This approach allows studying the interactions between membrane and peripheral proteins and also compartmentalized biochemical processes. For instance, Total Internal Reflection Fluorescence (TIRF) microscopy has been employed in order to investigate the detachment process of two types of cells from PEG-based thermoresponsive polymer coatings based on oligo(ethyleneglycol) methacrylate (OEGMA) and 2-(2-methoxyethoxy)ethylmethacrylate (MEO_2MA). Although the thin gold layer required for covalently anchoring the poly(MEO_2MA-*co*-OEGMA) polymer to the substrate affects image quality, TIRF microscopy yields reproducible, quantifiable, and time-resolved data on the detachment of the cells.[150] Besides this, the conformations of structurally well-defined polymers anchored to fluid lipid membranes were probed using Fluorescence Interference Contrast Microscopy (FLIC).[151] FLIC imaging is able to reveal the orientation of the molecules at solid-supported membranes, i.e., whether they project out from the membrane or lie flat.

Confocal Laser Scanning Microscopy (CLSM) has been also extensively used to visualize immobilized fluorescent nanostructures and nanoassemblies on surfaces as well (Figure 4C).

3.7. Electrophysiology

Electrophysiological approaches enable the study of functional activities of ion channels, porins, and other pore-forming molecular complexes at the single molecule level in the artificial membranes.[121] For example, the physical properties of OmpF such as gating kinetics and conductance were investigated after the reconstitution of the membrane protein into giant unilamellar vesicles (GUVs). Since OmpF is the main entrance for beta-lactam antibiotics the translocation processes of antibiotics were also monitored.[152] Different channel proteins such as α-haemolysin, OmpG, and alamethicin were incorporated into PMOXA-PDMS-PMOXA-based block copolymer membranes with thicknesses of 5.7 or 9 nm and preserved the pore function, with the exception of αHL into the 9 nm polymer membrane.[153] The presence of the large αHL head prevented the hydrophobic β-barrel from inserting further into the thick membrane (Figure 4B). Electrophysiology allows to precisely identify the physical and chemical factors which are required or support the channel functionality. Indeed, there are ion channels, such as ligand-gated channels, which require the presence of specific molecules-activators (ligands) in order to induce channel opening, while others require the synchronized presence of a number of molecular components or physical factors (temperature, pressure) in order to stimulate channel openings. However, the main challenge of this technique relies on the protein part, especially in the effectiveness of

the reconstitution procedure in membranes as well as protein purification, incorporation, and folding within the membrane. Electrophysiology provides crucial information on the size of a channel and its function such as channel opening and closing upon ligand binding, pH-induced conformational changes, ion selectivity or substrate specificity. Furthermore, measurements of the electrical properties of planar solid-supported membranes based on PDMS-*b*-PMOXA amphiphilic diblock copolymers showed that a potassium channel MloK1 was successfully inserted into polymer membrane and the biomolecule retained its functionality.[59] In addition to that, electrochemical impedance spectroscopy (EIS) represents a powerful tool for the investigation of the of charge transfer rate and charge transport processes occurring in electrochemical systems. Thus, it is widely used for the characterization of conducting polymer films and membranes and it provides important information on the membrane condition and sensor functionality EIS has been extensively used also to investigate the influence of membrane components (ion exchangers,[154] ionophores,[155] plasticizers[156,157]) and to understand adsorption and fouling mechanism of proteins and surfactants.[158–161]

4. Application of Functional Surfaces

Polymeric membranes decorated with biological molecules can be employed within several application fields that range from biomedical sensors, biocomputing devices, cell manipulation to high selectivity water filtration devices and batteries.

4.1. Biocomputing

Molecular systems capable of performing Boolean logic gates (AND, OR, IF) are the building blocks for biomolecular computing (biocomputing).[164] Biocomputing devices rely on the possibility to use nanoparticles, or biomolecules as processors for logic gates to perform complex operations due to selective molecular recognition and biochemical processes to yield single output signals.[164] Such high reaction and output precision, helps to develop devices are expected to provide "on demand" medical care, monitoring, and even new biopolymer synthesis.[165,166] The main issues that are encountered during the computation process are related to leakage, crosstalk, and scale up. Tethering DNA strands to lipid membranes has shown a drastic reduction of the aforementioned drawbacks, allowing much higher precision sensing.[167] Nanoparticles have also been employed in hybrid lipid/polymer membranes, additionally functionalized with DNA strands. The membrane is thus so designed that it can selectively interact with molecular information present in the external environment acting as the input. The consequent output, which relies on the intrinsic mobility of the membrane, is the regulated assembly of the confined NP within the membrane (**Figure 5**A).[162] Polymer membranes are also employed as static scaffold to implement bioelectrochemical systems. In this case, a polymer brush containing electroactive moieties reacts with the enzyme via pH changes, in situ directed by the enzyme itself.[13] A combination of free inputs in solution, based on simple chemical molecule (glucose) and an enzyme (glucose oxidase, GOx), triggers AND gates, which lead to TRUE and FALSE outputs.[168] Immobilizing the same systems on a hydrogel matrix supports increases the amount of logical gates accessible, which include AND-RESET, OR, OR-RESET.[169] This is possible through local changes in pH of the gate environment that can be read in sequential manner regulating thence the output. The combination of stimuli responsive polymers on a gold support and NADH coenzyme has allowed the use of a ternary input (0,1,2) instead of the classic binary (0,1) which enhances the accuracy of the reading and output.[170] In this case, poly(*N*-isopropylacrylamide-*co*-3-aminophenylboronic acid) (P(NIPAM-*co*-APBA)) was accountable for the amplification of the cyclovoltammic (CV) signal since it allowed direct oxidation of NADH on the Au surface on which it was electropolymerized.[170] One of the downsides of this area of

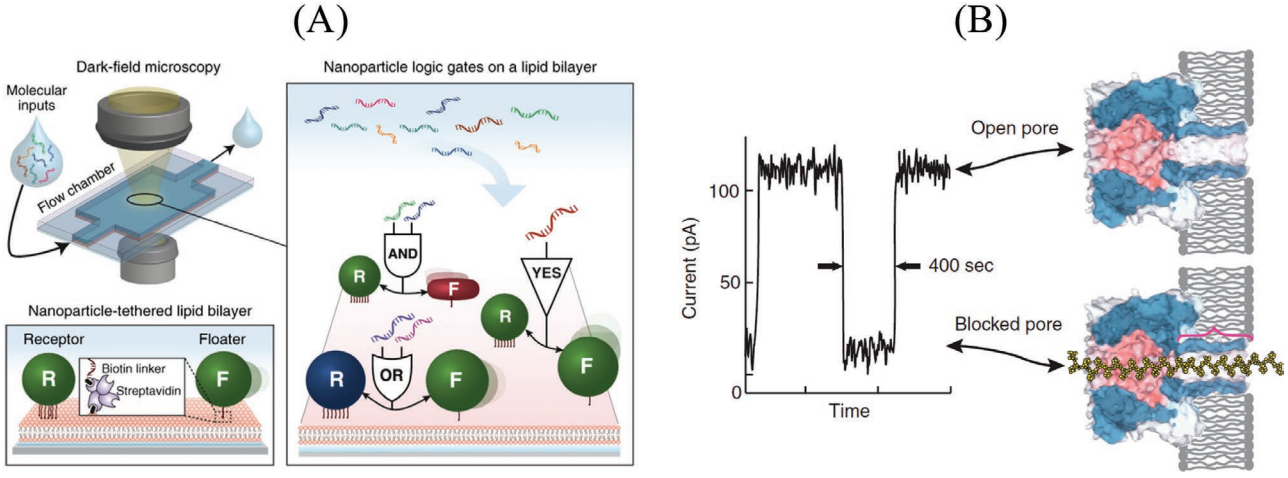

Figure 5. A) Schematic drawing of single-nanoparticle logic computation monitored by dark-field microscopy. DNA-modified nanoparticles are tethered on a bilayer with the immobile Receptor (R). Upon the input of DNA, R–F pairs function as Boolean logic gates leading to assembly or disassembly between two nanoparticles and subsequent color combinations. Adapted with permission.[162] Copyright 2019, Wiley-VCH. B) α-HL pore with typical ionic current amplitude sequencing signals when open or blocked by nucleotides. Reproduced with permission.[163] Copyright 2008, Springer Nature.

research is the little attention put on the engineering part of the devices. In fact most of these processes are performed manually, or in the best of cases through a peristaltic pump.[171] To overcome this drawback, an PMMA PDMAEMA brushes modified ITO electrode coupled to deposited DNA strands has been developed, in which the logical processes go through the DNA strands, increasing the degree of automation.[171] Since the output optimization had been under strong focus for an error-free signal from proteins, biocomputing systems can play a fundamental role for the next generation of biosensing devices.[172]

4.2. Cell Manipulation

Polymeric membranes and polymer decorated surfaces can serve both as cell adhesion promoters and as anti-fouling membranes.[173] Tuning their chemical composition can lead to "on demand" adhesion and detachment of cells, by introducing a stimuli responsive polymer or polymer/lipid membranes. Directing cell migration plays a fundamental role in wound regeneration and in general tissue formation, and can prevent cancer cells from spreading.[174] A change in hydrophilicity or mechanical properties leads to a different membrane-cell interaction, which opens the pathway to a controlled cell manipulation system. For instance, cell attachment on RGD decorated PNIPAM brushes was promoted by the peptide sequence on the brush, whereas detachment was triggered by polymer swelling at ambient temperatures.[175] It has been shown that at the physiological temperature, at which PNIPAM is hydrophobic, that cells attach to the membrane, but once the temperature drops below the LCST of the polymer (32 °C), the hydration of the chain promotes cell detachment.[176] Besides, hydrophilic spacers, such as PEG chains between the solid support and the membrane lead to accelerated cell detachment from the membrane. Not only ON/OFF behavior can be tuned by surface chemistry, but also directed migration can be obtained when designing anisotropic membranes.[174] PE multilayers composed of poly(sodium-4-styrenesulfonate) (PSS) and poly(diallyldimethylammonium) chloride (PDADMAC) were treated with a gradient solution of NaCl to induce anisotropic swelling.[174] These PE multilayers outperformed the homogeneous ones in the speed at which the adhered cell would migrate from one point to another. The design of anisotropic polymeric membranes that bear a gradient able to direct cell migration is generally difficult to obtain, and generally hampers the scale-up of the device.

4.3. Biosensors

As a concept, a biosensor incorporates a biological sensing moiety within a device connected with a transducer.[177] When it comes to polymer made biosensors, these are based on biomolecules embedded within the polymer support and which react to a certain physical stimulus or presence of a specific biomolecule by giving an output signal, such as a change in intrinsic fluorescence. The most common biosensing membranes are designed as polymer bilayers that embed channel proteins for specific sensing. When designing a biosensing membrane, the sensing protein plays a fundamental role in the fine-tuning of the selectivity and sensitivity of the membrane. For instance, when membrane embedded α-Hemolysin (α-HL) is commonly used for DNA sequencing.[178] Since α-HL has an extremely narrow pore (1.4 nm in diameter), the bases of the DNA strand passing through it can be read by a change in ion current characteristic for that base (Figure 5B).[125] However, due to its pore size, it encounters difficulties in selectivity for larger sequences, as for shorter ones. To overcome this issue, the pore size can be modulated via chemical modification. The introduction of barrel mutants supported by cyclodextrin adapters enabled the correct reading of four mononucleotides, dramatically increasing the sensitivity of these kinds of membranes.[179] The channel can be changed also by changing the channel proteins employed within the membrane. For example, OmpF has shown to be a promising channel protein in the design in sensing devices for its high versatility, as it is more resistant to solvent, pH, and temperature compared to other channel proteins. The most striking characteristic is its relatively large channel size with a cutoff of 600 Da, which can be opened and closed on demand with a pH switch.[180] Molecular printing combined with Au nanoparticles has been shown to increase the sensing capacity of functional membranes, as in the case for a membrane bearing creatine deaminase, for which the Au NP allow a higher selectivity in the detection of conductance changes due to enzymatic activity.[181] Along with proteins and small biomolecules, nanoassemblies on surfaces have shown good applications for the sensing of pH changes.[182,183] Sugar sensing is also possible when encapsulating ribitol dehydrogenase enzymes within a PMOXA-PDMS-PMOXA polymersome.[78] The permeability was favored by introducing glycerol facilitator (GlpF) on $PMOXA_6$-*b*-$PDMS_{42}$-*b*-$PMOXA_6$ based polymersome membranes and showed high sensitivity toward sugars, in an external concentration of Ribitol, taken as the model sugar, of 1.5-9 mM. The nanoreactors, although subjected to chemical transformation occurring within their cavity retained their shape once immobilized on the surface.[78] Furthermore, host guest chemistry nanoassemblies have shown good potential for small molecule sensing.[182] In this case, a glutamate biosensor was designed combining an eugenol film with β-CD, in which the high permeability to hydrogen peroxide and low permeability of ascorbic acid were used in the sensing approach. All the reported examples have been tested in vitro, thus involving higher fluid volumes, longer signaling times, and manual preparation. Even though polymer-based biosensors are generally low cost, the signaling is not always quantitative, and the determination of the amount of analyte is sometimes challenging.

4.4. Antimicrobial Surfaces

One of the main challenges in the field of medicine is to develop materials with resistance to microbial growth, which leads to compliance and infections.[109,184] In the past years, the development of surfaces and composite membranes for antimicrobial applications has been under strong focus. Three approaches have resulted in the most successful so far: the use of antibacterial agents released by a gel, solid-supported

antimicrobial peptides or antimicrobial nanoparticles and micropatterned surfaces. When it comes to micropatterning, antifouling and fouling release properties can be reached within the same membrane. In fact, the distribution of poly(allylamine hydrochloride)/(poly(styrenesulphonate) brushes in a checkboard pattern with PDMS brushes, ensured that the first ones promoted antifouling activity with a 70–93% bacteria reduction and the latter promoted the fouling removal.[185] Fine tuning the chemistry of the polymer brush plays also a fundamental role in their bactericidal activity when the brushes are coupled with antimicrobial peptides.[186] Membranes composed of zwitterionic brushes decorated with antimicrobial peptides outperformed their neutral yet hydrophilic counterparts.[187] Nonactive solid-supported polymer brushes play a role in antimicrobial applications, when combined with active elements such as antibiotic, antimicrobial peptides and nanoparticles. For instance, poly(sulfobetaine methacrylate) and poly(carboxybetaine methacrylate) brushes were used to embed silver nanoparticles which allocate the antibacterial activity.[188] The combination of antimicrobial NP and zwitterions shows outstanding antimicrobial activity of the membrane, where the bacterial population was reduced by 100% after 6 h incubation time. Lastly, supramolecular assemblies can also be immobilized on a solid support and carry antimicrobial effects. solid-supported nanoreactors perform as a catalytic site to produce specific compounds to kill bacteria. An elegant example is an ABA triblock composed of PMOXA-PDMS-PMOXA which self-assembled into polymersomes and was immobilized on a surface, was able to efficiently produce antibiotics (**Figure 6**A).[152] The nanoreactors were characterized by reconstituted OmpF channel protein for the selective diffusion of small molecules in their membrane, and contained in their cavity penicillin acylase able to react with the antibiotic precursor which was free is solution and that would diffuse within the vesicle, and release to the outside the antibiotic. Since OmpF allows the gradual diffusion of the precursor molecules within the cavity of the polymersome, this leads to the continuous activity of the enzyme, and inhibition of bacterial growth up to 7 days.[152] The main issue encountered in the formation of antimicrobial surfaces is their poor performance compared to antibiotics, and the sometimes formation of side products which might have a toxic effect in vivo.[189] For this reason, pairing antimicrobial nanostructures with surfaces increases the number of active elements and is less subject to fouling when in presence of an antifouling polymer support.

4.5. Membranes for Water Filtration

In recent years, bioinspired membranes for filtration have caught major attention due to their availability and efficiency. The combination of commercial polymers with low cost biomolecules ensures an easy scale-up for industrial applications. The major application fields for filtration membranes consist in desalination and ultrafiltration. Desalination works usually on reverse osmosis, in which the membrane is used as a filter to generate desalinated water under pressure. Desalination membranes do not require high mechanical stability, but rather high activity in retaining ions. The selectivity can be achieved by multiblock polymers, bearing functional moieties for salt retention.[191] Self-assembly of polymer/ion complex is exploited on solid supports, as in the case of a PN-supported poly(ethyleneiminie) complexed with Ag^+ ions, which display high efficiency in dye desalination.[192] Moving from synthetic supports to natural polymers, such as cellulose acetate, allows also to obtain membranes for salt rejection.[193] In this case, a bio-based composite material composed of cellulose acetate and carbon nanoparticles is a good example for high performing renewable materials. Homopolymeric cellulose can also serve as high performing desalination membrane.[194] In detail, introducing nitrophenol as a plasticizer and its subsequent removal leads to an internal rearrangement of the

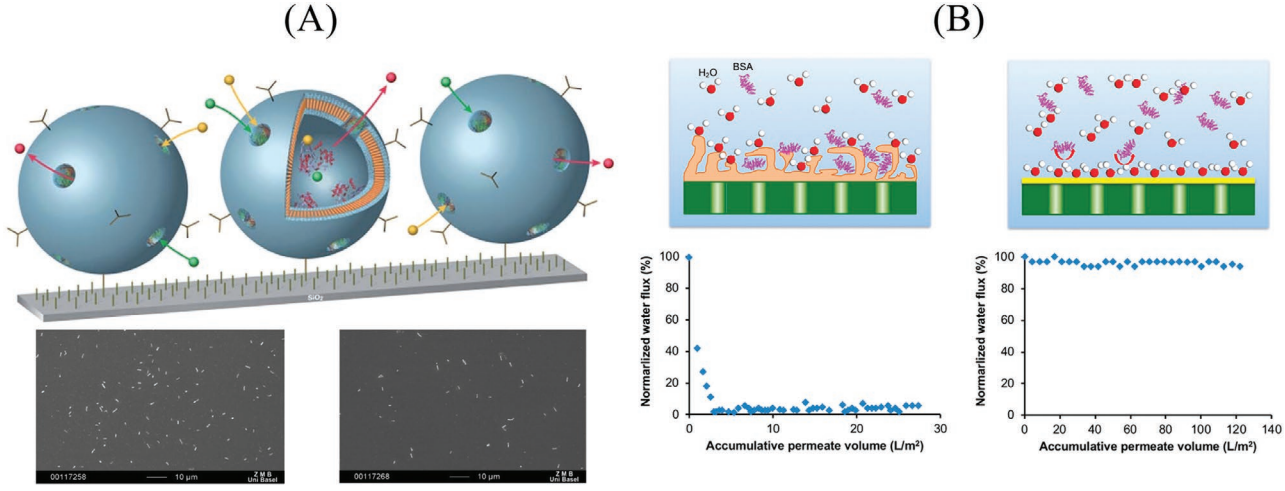

Figure 6. A) Representation of immobilized nanoreactors functional to synthesize antibiotics. Green and yellow are educts and red is the synthesized antibiotic. SEM micrographs of attached *E. coli* on silicon surface (left) and active nanoreactors (right). Adapted with permission.[152] Copyright 2014, The Royal Society of Chemistry. B) Comparison of polyamide membranes against organic fouling fabricated with two different polymerization approaches. The filtration behavior is assessed by bovine serum albumin (BSA) with an unmodified membrane leading to rapid deposition of BSA (left) and no deposition (right). Adapted with permission.[190] Copyright 2019, American Chemical Society.

microcrystallites, which dramatically enhance selectivity while retaining the membrane's mechanical stability. Aquaporines are a molecular channel already used for water filtration and desalination by embedding them in self-assembled block-copolymer membranes.[195,196] Using amphiphilic peptides to stabilize the pore improved the self-assembly and the stability of the membrane.[197] These membranes are however prone to be colonized by bacteria and biological colonies, and the use of antifouling agents such as chlorinated polluting agents is required.[198] Apart from desalination, ultrafiltration is a highly popular application for biomimetic membranes. As for the desalination, architectures range from layer-by-layer (LBL) assembled alginate-Cu^{2+} assembled membranes to composite (LBL) membranes.[199,200] PS-*b*-PMMA-*b*-P*t*BA triblock were used as successful ultrafiltration membrane material and serve as example for self-assembled nanostructures that lead to selective filtration.[201] The horizontal self-assembly lead to the formation of nanochannels through the membranes, and their size can be tuned by swelling and deswelling of the external block. Block polymer can also be used as additives to enhance the ultrafiltration effectivity, as in the case of PEO-PMMA added to poly(vinylidenefluoride) membrane.[202] Membranes composed of self-assembled block polymers mimicking bio patterns show great performance in the filtration of nanoparticles while having important antifouling properties.[203] Filtration membranes were also obtained by using solid-supported zwitterion polymers or biomolecules-based zwitterions such as glutamic acid and lysine, which provide antimicrobial properties and thus find wide applications for reverse osmosis membranes and water purification (Figure 6B).[190,204]

5. Current Limitations and Future Outlook

Solid-supported planar synthetic membranes represent a class of materials that interacts well with biomolecules and proteins, paving the way to hybrid bioinspired materials that find applications in various fields. Membranes composed of block/co-polymers have the main advantage to be extremely versatile in terms of chemical functionality, length, flexibility, and relative polarity. This enables the formation of self-assembled planar membranes characterized by a broad range of functionality and anchors for biomolecules and proteins, adjustable membrane thickness and lateral diffusion properties. Moreover, polymer-based membranes outperform their lipid-based counterparts in terms of mechanical stability, creating rather robust systems that allow the investigation of transmembrane proteins under harsher conditions.[18]

The transfer of the membrane on a solid support can be done covalently via grafting techniques, namely grafting from and to, whereas the non-covalent introduction of a membrane is obtained either or the transfer of a previously self-assembled membrane is obtained via LB and LS techniques. Carefully choosing which technique suits the application best is the key to promote a synergistic effect between the synthetic membrane and the biomolecule, producing high performing materials, specifically for biomedical, biosensing, and antifouling applications. Besides planar membranes, self-assembled nanostructures tethered on surfaces represent a niche in surface chemistry, which however presents a wide scope of applications, from synthetic biology, sensing applications, and catalysis. Tethered vesicles on surfaces constitute active surfaces that prevent uncontrolled delivery since the active molecules are entrapped within the cavity of the polymersomes and are not subjected to denaturizing elements. The coupling of polymers tethered on a surface with biomolecules however lacks to this point of standardization and slows down the scale-up process on an industrial level.[205] This is due to the fact that often it is difficult to control the polymer assemblies at the nanoscale, thus extremely small systems are generally used to facilitate the desired outcome. In the same way, reconstitution of proteins within the membranes is still limited on the small scale and presents too many limitations in the scale-up.[13] The presence of biomolecules also triggers issues in sterilization, as harsh chemicals or high temperatures used to annihilate the eventual pathogens can have a denaturizing effect on the biomolecules and thus on the functionality of the surface.[206] UV-triggered degradation is also within the spectrum of improvement that these membranes have to undergo, and the overall stability of the surfaces needs further improvement due to the eventual formation of unwanted and toxic byproducts.[207] Nevertheless, the functions provided by biological and synthetic membrane proteins play a key role in developing artificial biomimetic structures. These important structures might constitute better engineering materials for membranes from the perspective of mechanical, chemical, and biological stability while providing many routes for functionalization and incorporation in to membranes through synthetic chemistry. If on one hand, the optimization route for synthetic membranes to increase their versatility and interplay with biomolecules, on the other one little has been done to design self-assembled planar membranes that are easily scalable for commercial use. The same scenario characterizes self-assembled nanostructures on surfaces, which are still in their infant stage of discovery, rather than already at the optimization level and in vivo studies. For this reason, further research has to be done in order to transfer the applications of this class of material into commercial and biomedical use.

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Conflict of Interest

The authors declare no conflict of interest.

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biomimetic, biomolecules, nanoassemblies, polymer membranes, polymer monolayers, self-assembly, surfaces

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